**Lecture 33: Electron transport, ATP synthesis (Oxidative Phosphorylation)**

(Anerobic metabolism will appear in lecture 34)

**Electron Transport:**

The energy captured in glycolysis, TCA cycle, and fatty acid oxidation is converted to a **proton gradient** across the inner mitochondrial membrane. *The energy stored in this gradient is used to produce ATP*. The source of this energy is the oxidation of NADH and FADH2:

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| **Pathway** | **NAD+/NADH** | **FAD/FADH2** |
| Glycolysis | Glyceraldehyde 3-phosphate dehydro. |  |
| TCA cycle | Pyruvate dehydrogenaseIsocitrate dehydrogenaseα-ketoglutarate dehydrogenaseMalate dehydrogenase | Succinate dehydrogenase |
| Fatty Acid Ox, | hydroxyacyl-CoA dehydrogenase | Acyl-CoA dehydrogenase |
| Within Pathway |  |  |
| Electron Transport |  |  |

In most organisms the electrons from these compounds are deposited on oxygen, reducing it to water. Note that the oxygen only serves as a final acceptor of electrons in this process, the actual **synthesis of ATP** is from a **proton gradient** across the inner membrane that is generated during the transfer of electrons to oxygen.

In many organisms other compounds besides oxygen can serve as electron sinks, allowing organisms to perform 'oxidative' phosphorylation in the absence of O2.

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| Oxidation of NADH | NADH → NAD+ + 2 e- + 2H+ | ΔG = -60 kJ/m. |
| Reduction of oxygen | 2e- + 2H+ + (1/2) O2 → H2O | ΔG= - 156 kJ/m. |
| **Tot. Reaction** | **NADH + (1/2) O2 → H2O + NAD+** | **-200KJ/mol** |

Key Components in Electron Transfer:

**1. Inorganic carriers of electrons**

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| 1. Iron-sulfur centers (e.g. succinate dehydrogenase)

 | b) Fe in heme – e.g. cytochrome C |

**2. Organic Carriers of electrons:**

a) Coenzyme Q. Coenzyme Q is a non-polar electron carrier that diffuses freely in the ***fluid*** mitochondrial membrane.

**Electron Transport: Gibbs Energy & Flow**

***Complex I*: NADH-CoQ oxidoreductase**

* **Four protons/2*e****-* are pumped from the inside (matrix) to the intermembrane space.

***Complex II*: Succinate-CoQ oxidoreductase**

* Succinate dehydrogenase of the citric acid cycle is part of this complex.

* Two electrons from FADH2 are transferred to CoQ via Fe-S clusters, generating CoQH2.
* **Does not pump any protons.**

***Complex III*: CoQH2-cytochrome c oxidoreductase**

* Transfers electrons from CoQH2 to cytochrome c one electron at a time.
* **Four protons are pumped/ 2*e****-.*

***Cytochrome C****:* Shuttles electrons from III to IV.

***Complex IV*: Cytochrome c oxidase**

* Accepts 4 × one *e-* at a time from cytochrome c.
* Donates a total of *four* electrons/O2.
* Site of oxygen reduction to water.

i) Produces 2 water molecules/O2 molecule.

ii) Pumps an additional **two protons/*2e****-.*

**Energy Stored in the Proton Gradient**

The energy 'stored' in a concentration gradient can be considered to consist of two parts (Reaction direction outside to inside).

i) The Gibbs energy due to a concentration difference across a sealed membrane. The Gibbs energy is:



The standard chemical potential (μ0) for the species ([X]) is the same on both the inside and the outside of the membrane, therefore:



This is the amount of energy that is released when one mole of [X] is brought from the outside to the inside.

ii) Movement of a charged particle through a voltage difference. The free energy associated with moving a particle of charge Z, through a voltage difference ΔΨ, is: ΔG=ZFΔΨ

* Z = the charge on the transported ion (+1 in the case of the proton)
* F is Faraday's constant, 96,494 C/mol. C=coulomb
* ΔΨ is the voltage difference across the membrane, in volts. This voltage difference is often referred to as the membrane potential.

The total Gibbs free energy is the sum of these two terms: 

**Example Calculation:** Typical values across the inner mitochondrial membrane are:

[H+]IN/[H+]out = 0.1 (pH=6.5 outside, 7.5 inside),

Δψ = -150 mV, i.e. a net positive charge on the outside of the membrane:



**ATP Synthesis:**

*ATP synthesis is attained by coupling the free energy of a proton gradient to the chemical synthesis of ATP. The enzyme that accomplishes this coupling is called* ***ATP-synthase*** *(also known as FoF1 ATPase)*

***3 H+ = 1 ATP synthesized***

**Structural Features:**

1. The Fo Complex

* Membrane-spanning, multi-protein complex.
* Responsible for coupling the movement of three protons to 120° rotations of the **γ-subunit**.

2. The F1 Complex

* Attached to Fo, it protrudes into the mitochondrial matrix.
* Composed of five different subunits: α3β3γδε
* The γ subunit is the shaft at the center of the α3β3 disk. **γ rotates 120o/3 protons.**
* The β subunits are asymmetric due to their interactions with the γ-subunit.
1. One conformation of the β subunit has very **low affinity** for both ADP and ATP.
2. One conformation of the β subunit has **high affinity for ADP and Pi**.
3. One conformation of the β subunit has **high affinity for ATP**.

**How the motor works**:

* Every time three proton move through the complex, the γ subunit rotates 120°.
* The rotation of γ subunit changes the conformation of the β-subunits such that the Gibbs energy of the bound ADP + Pi becomes higher than the energy of ATP, thus ATP forms spontaneously from the bound ADP and Pi.
* The newly-formed ATP is released with the transport of three additional protons.
* The actual synthesis, or formation of the bond between ADP and PI, is catalyzed by conformational changes of the β-subunit that occur as a consequence of the rotation.
* Since all three β subunits are functioning at the same time, the transport of 9 protons in a complete cycle produces 3 ATP.

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| **NADH** | **~10 protons pumped** | **~ 3 ATP** |
| **FADH2** | **~6 protons pumped** | **~2 ATP** |